Dynamic metabolic flux analysis using spline parameterizations

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1 Introduction

In general, the applicability and reliability of existing predictive microbiology models, which are mostly black-box models, under realistic conditions can definitely be improved by unraveling the underlying mechanisms and incorporating intracellular (microscopic) information, effectively taking a look inside the black box. Following a systems biology approach, the link between the intracellular fluxes and the extracellular measurements can be established by techniques of metabolic flux analysis (MFA). This is a linear technique for estimating unknown intracellular fluxes based on macroscopic measurements, which is typically used in a steady-state approach.

2 Methodology

In this work the MFA framework is extended to a dynamic form. Different techniques for dynamic MFA are already described in literature, but they all have some drawbacks. One possibility is to just connect flux solutions from steady-state analyses at different points in time [1]. This technique just uses standard MFA-methodology, but does not give a fully dynamic view of the metabolism. In another technique, the unknown flux profiles are parameterized as piecewise linear functions, and the resulting linear dynamic model is solved analytically, after which the flux profile parameters can be found using least-squares minimization [2]. This method has two drawbacks: it is only usable for linear dynamic systems, and the flux profiles are not smooth. To overcome these drawbacks, two features are included in the proposed methodology: *(i)* the flux profiles are parameterized as higher order B-splines, and *(ii)* a state-of-the-art NLP solver with automatic differentiation is used to solve the resulting dynamic parameter estimation problem by a collocation approach.

3 Methods

The flux profiles are described as B-splines with free knots. The estimation of these free knot locations is a highly nonlinear problem. To make sure that global mimima are found, a sequential parameter estimation approach is used, in which new knots are added using knot insertion until a goodness-of-fit criterion is satisfied. The dynamic parameter estimation problems is solved by discretizing the differential states via a direct collocation scheme. The resulting nonlinear program is solved with CasADi, a software package for automatic differentiation and dynamic optimization [3]. Gradients, Jacobians and Hessians are automatically generated in CasADi, and these are then used to solve the NLP using the optimization routine IPOPT [4].

4 Results

Two examples in which the described methodology is used are illustrated. A first example is a toy network with three states and two unknown fluxes. A second example is a real-life case-study with 11 states and 7 unknown flux profiles. In both cases, the unknown dynamic fluxes are successfully estimated, including confidence intervals using a Monte Carlo approach.

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